

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: In vitro diagnostic test kit for *HER2* gene amplification in formalin-fixed, paraffin-embedded (FFPE) tissue sections using Chromogenic In Situ Hybridization (CISH)

Device Trade Name: *HER2* CISH pharmDx™ Kit

Applicant's Name and Address: Dako Denmark A/S
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Denmark

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P100024

Date of FDA Notice of Approval: November 30, 2011

Expedited: Not applicable.

II. INDICATIONS FOR USE

HER2 CISH pharmDx™ Kit is intended for dual-color chromogenic visualization of signals achieved with directly labeled in situ hybridization probes targeting the *HER2* gene and centromeric region of chromosome 17. The Kit is designed to quantitatively determine *HER2* gene status in formalin-fixed, paraffin-embedded breast cancer tissue specimens. Red and blue chromogenic signals are generated on the same tissue section for evaluation under bright field microscopy. The CISH procedure is automated using Dako Autostainer instruments.

HER2 CISH pharmDx™ Kit is indicated as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered. Results from the *HER2* CISH pharmDx™ Kit are intended for use as an adjunct to the clinicopathologic information currently used for estimating prognosis in stage II, node-positive breast cancer patients.

This kit is for *in vitro* diagnostic (IVD) use only.

III. CONTRAINDICATIONS

The contraindications can be found in the *HER2* CISH pharmDx™ kit labeling.

IV. **WARNINGS AND PRECAUTIONS**

The warnings and precautions can be found in *HER2* CISH pharmDx™ Kit labeling.

V. **DEVICE DESCRIPTION**

HER2 CISH pharmDx™ Kit is a dual-color chromogenic *in situ* hybridization (CISH) assay used for slide-based, bright field microscope identification and quantification of *HER2/neu* gene amplification in formalin-fixed, paraffin-embedded (FFPE) tissue sections. *HER2* CISH pharmDx™ Kit contains all specific reagents required to complete a CISH procedure. In the procedure, the targets (*HER2* and CEN-17) are labeled by *in situ* hybridization using fluorescent-labeled DNA and PNA probes. Before signal evaluation the fluorescent signals are converted to chromogenic signals in an immunohistochemical reaction that includes blocking of endogenous peroxidase activity, incubation with CISH Antibody Mix and subsequently visualization of the signals by incubation with chromogens.

The *HER2*/CEN-17 Probe Mix consists of a mixture of Texas-Red labeled DNA cosmid clones that cover 218 kb of the chromosomal region that includes the *HER2* gene plus a mixture of fluorescein-labeled (PNA) probes targeted towards the centromeric region of chromosome 17. The probes are pre-mixed in hybridization buffer for ease of use. Unlabeled PNA probes are also included to suppress sequences contained within the target loci that are common to other chromosomes.

The CISH Antibody Mix includes anti-Texas Red conjugated with alkaline phosphatase (AP) that convert the Texas Red-labeled signals (*HER2*) to red chromogenic signals and anti-FITC conjugated with horseradish peroxidase (HRP) that convert the FITC-labeled signals (CEN-17) to blue chromogenic signals.

Upon specific hybridization at the two targets and subsequent conversion to chromogenic signals a red signal is seen at each *HER2* gene and a blue signal is seen at the chromosome 17 centromere. In each cell, the copy numbers of *HER2* and CEN-17 are enumerated. The presence of amplified *HER2* is determined by the ratio of the average copy number of *HER2* and CEN-17. Gene amplification is defined as a *HER2*/CEN-17 ratio ≥ 2.0 , while *HER2*/CEN-17 < 2.0 indicates non-amplification.

VI. **ALTERNATIVE PRACTICES AND PROCEDURES**

There are several alternative methods for the detection of *HER2* gene amplification. Devices utilizing fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) methodologies for gene amplification determination in human breast cancer tissue specimens are commercially available. This is in addition to immunohistochemistry (IHC), another alternative procedure for detection of gene product overexpression in human breast. A physician should consider these alternatives and select the appropriate method to be used.

VII. MARKETING HISTORY

HER2 CISH pharmDx™ Kit has been available in the following European Union countries - Switzerland, Norway, Lichtenstein and Iceland since June 2010 and in Canada since September 2010. This device has not been withdrawn for any reason related to safety and effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

HER2 CISH pharmDx™ Kit is intended for in vitro diagnostic use only. As with any in vitro diagnostic test, there are potential risks associated with incorrect result interpretation. A false positive test result would likely assign patients to receive a more aggressive therapy, possibly exposing the patient to serious side effects and, in rare cases, death. Alternatively, a false negative test result may exclude a patient who might benefit from therapy, potentially resulting in a poor clinical outcome.

IX. SUMMARY OF NON-CLINICAL STUDIES

Analytical performance testing was performed to evaluate the safety and effectiveness of *HER2* CISH pharmDx™ Kit. The validation included analytical sensitivity, analytical specificity, locus specificity, robustness, reproducibility, repeatability and stability testing.

A. Laboratory Studies

1. Analytical Specificity and Sensitivity

a. Hybridization Efficiency

To verify that the *HER2* CISH pharmDx Kit specifically detect their target sequences, the *HER2* CISH pharmDx probe mix was used to stain human metaphase spreads.

The *HER2* DNA probes in the *HER2*/CEN-17 Probe Mix have been end-sequenced and mapped to confirm total coverage of 218 kb including the *HER2* gene. The CEN-17 PNA probes in the *HER2*/CEN-17 Probe Mix have been tested individually and in combination to confirm their specific hybridization to the centromeric region of chromosome 17.

A total of 250 metaphase spreads were evaluated for specific hybridization of the *HER2* DNA and CEN-17 PNA probe mixes. This study was done according to the instructions provided in the labeling. To ensure that there was no cross-hybridization to chromosomes other than chromosome 17, studies were performed on metaphase spreads according to standard Dako QC procedures. In all 250 cases the hybridization was specific for chromosome 17. No cross-hybridization to loci on other chromosomes was observed in any of the 250 cases.

b. *Analytical Sensitivity (quantitative)*

The analytical sensitivity for *HER2* CISH measures the assay's ability to detect the target substances (*HER2*/CEN-17). The analytical sensitivity of *HER2* CISH pharmDx™ Kit probes was determined using 18 normal human breast tissue specimens.

All specimens showed a *HER2*/CEN-17 ratio within the preset acceptance criteria for normal breast tissue (0.97-1.09) and with consistent results between FISH (previously approved in P040005) and CISH. Assay sensitivity showed that the assay performs according to specifications (within the preset acceptance criteria) with an estimated mean ratio of *HER2*/CEN-17 of 1.03 (S.D. = 0.04) (normal breast tissue has an estimated *HER2*/CEN-17 ratio between 0.8-1.4) and there is consistency between *HER2* CISH and *HER2* FISH measurements

c. *Analytical Specificity*

Studies were performed to assess the analytical specificity for *HER2* CISH and to measure the assay's ability to solely identify target substances *HER2*/CEN-17 without interference from other substances. Stained slides were evaluated for presence of signals in the absence of probes or antibodies with complete FISH and CISH staining used as reference. Analytical specificity was tested on 18 normal human breast tissue specimens. Assay specificity showed that there is no non-specific binding of CISH Antibody Mix or Red and Blue Chromogen that result in signals visible in CISH upon omission of Probe Mix and Antibody Mix.

2. Assay Robustness Studies

a. *Tissue Thickness*

The effect of section thickness on the performance of *HER2* CISH pharmDx was studied. A total of 10 serial sections of breast carcinoma (two specimens representing \pm *HER2* gene amplification on each slide) with different thickness (3-7 μ m) were tested. Repeatability of *HER2*/CEN-17 ratio evaluated on section of different thickness showed a coefficient of variance of 3% for the non-amplified specimen and 6% for the amplified specimen, within the preset acceptance criteria (see Table 1 1). This is within the interval observed for sections of equal thickness. Based on the studies, the recommended thickness for sections is 4 -6 μ m.

Table 1: Section thickness

Summary of Safety and Effectiveness Data (SSED)

Duplicate measurements with varying slide thickness	Ratio (<i>HER2</i> /CEN-17) Non-amplified samples	Ratio (<i>HER2</i> /CEN-17) Amplified samples
3 μm	1.06	9.81
3 μm	1.00	10.28
4 μm	1.03	9.57
4 μm	1.06	10.33
5 μm	1.00	11.38
5 μm	1.06	9.82
6 μm	1.00	9.47
6 μm	1.03	10.33
7 μm	1.09	9.35
7 μm	1.09	10.16
%C.V.	3	6

b. Incubation Time and Temperature

Robustness of *HER2* CISH pharmDx™ Kit assay was tested by varying time and temperature for incubation with CISH Antibody Mix, Red Chromogen Solution, Blue Chromogen Solution and counterstaining. No significant difference in results was observed at the following experimental conditions (unless otherwise indicated):

- **Antibody Mix** incubation time: 25, 27, 30, 33 and 35 minutes. 25 minutes incubation with Antibody Mix resulted in a loss of red signal intensity while no significant differences were observed at all other time points. Recommended incubation time is 30 minutes.
- **Red Chromogen** Solution incubation time for 8, 9, 10, 11 and 12 minutes. Recommended incubation time is 10 minutes.
- **Blue Chromogen** Solution incubation time for 8, 9, 10, 11 and 12 minutes. Recommended incubation time is 10 minutes.
- **Counterstaining** incubation time for 4, 5 and 6 minutes and concentration at 1:4, 1:5 and 1:6 dilutions with a recommended incubation time of 5 minutes at a 1:5 dilution.

The robustness of the steps including pre-treatment, denaturation/hybridization and post hybridization have been tested previously as part of the *HER2* FISH pharmDx™ Kit assay development. No significant difference in results was observed at the following experimental conditions (unless otherwise indicated):

- **Pre-treatment** at 7, 10 and 13 minutes combined with each of the temperatures 89, 92 and 95-99 °C. Recommendation is 10 minutes at ≥ 95 °C.

Summary of Safety and Effectiveness Data (SSED)

- **Pepsin** incubation times of 2, 5, 10, 15 and 18 minutes at room temperature. Recommendation is 5-15 minutes (depending on tissue fixation).
- **Denaturation** temperatures of 72, 77, 82, 87 and 92 °C were tested. Recommendation is 5 minutes at 82 °C.
- **Hybridization** time of 17 hours combined with each of the temperatures 40, 45 and 50 °C and hybridization times of 10, 12 and 14 hours at a temperature of 45 °C. Recommendation is 14-20 hours at 45 °C.
- **The stringent wash** was tested for 10 minutes at 60, 65 and 70 °C and for 5, 10 and 15 minutes at 65 °C. Stringent wash for 10 minutes at 70 °C, and stringent wash for 15 minutes at 65 °C resulted in loss of signals, whereas no significant difference in results was observed at the other time/temperature combinations. Recommendation is 10 minutes at 65 °C.
- **Dilution of Stringent Wash Buffer** was tested: 1:10, 1:15, 1:20, 1:30 and 1:40. The 1:40 dilution of Stringent Wash Buffer resulted in loss of signals, whereas no significant difference in signal intensity was observed at the other dilutions. Recommendation is 1:20 dilution.

c. Stability Testing

Results of real-time stability studies indicate that *HER2* CISH pharmDx™ Kit is stable for 10 months when stored per instructions in the package insert, i.e., 2-8 °C in the dark.

The stability studies included transport simulation (48 hours, 22 °C), 20 minutes working stability of the Red Chromogen Solution (at room temperature) and 8 days working stability of the Blue Chromogen Solution when stored at 2-8 °C in the dark and on-board stability for 30 hours (Ready to Use reagents). The performance of the kits was measured on tissue specimens according to the device labeling.

d. Repeatability

Repeatability of the *HER2*/CEN-17 ratio was investigated using 9 different FFPE breast carcinoma specimens with different *HER2* gene status. Consecutive sections of each specimen were tested three times under the same conditions (in the same run) and the *HER2*/CEN-17 ratio was determined. Repeatability of *HER2*/CEN-17 ratio showed a coefficient of variance between 1% and 7% (within the preset acceptance criteria) with a tendency for the higher values found for the amplified specimens (see Table 2). Repeatability on consecutive sections of breast cancer specimens with different thickness (3-7 µm) was tested with *HER2* CISH pharmDx™ Kit. The coefficient of variance of the *HER2*/CEN-17 ratio in this study was found to be 3-6%, i.e. in the same range as for tissue of equal thickness.

Summary of Safety and Effectiveness Data (SSED)

Table 2: Repeatability (intra-run)

Specimen ID	Ratio (<i>HER2</i> /CEN-17) Repeat 1	Ratio (<i>HER2</i> /CEN-17) Repeat 2	Ratio (<i>HER2</i> /CEN-17) Repeat 3	%CV
57210	3.12	3.33	3.23	3.0
57316	1.02	1.03	1.04	1.0
57342	1.00	1.09	1.05	4.0
54573	1.15	1.10	1.18	4.0
57240	1.03	1.05	1.03	1.0
57264	1.06	1.05	1.13	4.0
57304	7.00	6.77	6.50	4.0
57314	8.50	8.21	8.33	2.0
57308	7.36	8.09	7.15	7.0

e. Reproducibility

Reproducibility – lot-to-lot

Lot-to-lot reproducibility demonstrates the degree of agreement between evaluations of *HER2*/CEN-17 ratio carried out using three different production lots of *HER2* CISH pharmDx™ Kit. Reproducibility was tested on nine different breast carcinoma specimens with different *HER2* gene status. *HER2* gene status for each specimen remained unchanged from lot-to-lot. The coefficient of variation of the *HER2*/CEN-17 ratio was found to be between 1-7% for non-amplified specimens and 7-12% for amplified specimens (see Table 3) within the preset acceptance criteria.

Table 3: Reproducibility (lot-to-lot)

Specimen ID	Ratio (<i>HER2</i> /CEN-17) Lot 1 (Lot A)	Ratio (<i>HER2</i> /CEN-17) Lot 2 (Lot B)	Ratio (<i>HER2</i> /CEN-17) Lot 3 (Lot C)	%CV
57308	7.36	7.48	8.33	7.0
57304	5.82	6.56	6.95	9.0
54573	1.13	1.23	1.22	5.0
57314	8.19	8.32	9.3	7.0
57564	1.15	1.02	1.05	6.0
57240	1.05	1.06	1.08	1.0
57210	3.55	3.79	3.0	1.2
57342	1.06	1.15	1.0	7.0
57316	1.09	1.02	1.0	5.0

3. Non-clinical Studies – External

f. Site-to-Site and Day-to-Day Reproducibility

The reproducibility study was a three-site, blinded, randomized study, using formalin-fixed, paraffin-embedded (FFPE) human breast cancer specimens with different levels of *HER2* gene amplification. Nine specimens were analyzed and scored on three non-consecutive days at three study sites. These nine specimens included three non-amplified specimens, three *HER2* IHC 2+ specimens, as determined by HercepTest™ according to the manufacturer's recommendations, and three amplified specimens.

Summary of Safety and Effectiveness Data (SSED)

Results for site-to-site and day-to-day reproducibility

Data for the *HER2*/CEN-17 ratios obtained from the reproducibility study are tabulated in Table 4.

Table 4: Site-to-Site and Day-to-Day Reproducibility - *HER2*/CEN-17 ratios using automated CISH.

Specimen	Type	Day	<i>HER2</i> /CEN-17 Ratio		
			US Site 1	US Site 2	Dako Internal
54573	Non-amplified	1	1.21	1.05	1.15
		2	1.05	1.16	1.17
		3	1.26	1.17	1.06
57264	Non-amplified	1	1.20	1.08	1.09
		2	1.23	1.31	0.98
		3	1.13	1.06	0.97
57316	Non-amplified	1	1.05	1.14	1.10
		2	0.97	1.15	1.04
		3	1.11	1.31	1.07
57210	IHC 2+	1	2.59	2.33	3.15
		2	2.63	2.29	2.94
		3	2.46	3.52	3.03
57240	IHC 2+	1	0.98	1.09	1.06
		2	1.26	1.16	1.03
		3	1.08	1.17	1.00
57342	IHC 2+	1	1.28	1.21	1.06
		2	1.19	1.13	1.00
		3	0.90	1.12	1.03
57304	Amplified	1	8.22	6.94	7.02
		2	6.37	7.12	6.62
		3	5.89	6.00	6.11
57308	Amplified	1	9.08	5.16	7.72
		2	8.50	7.60	7.41
		3	8.55	6.48	6.76
57314	Amplified	1	7.64	6.33	8.26
		2	6.68	7.12	8.92
		3	8.24	5.96	8.32

The *HER2*/CEN-17 ratios were analyzed using a variance component model where estimated CVs were determined from log-transformed *HER2*/CEN-17 ratios. This resulted in CV estimates just below 10% for non-amplified specimens, at 12% for IHC 2+ specimens and close to 15% for the amplified specimens (Table 5). The measurements made on

Summary of Safety and Effectiveness Data (SSED)

different sites and different days seem to differ only slightly more than putative measurements made the same day at the same site (the residual error).

Table 5: Variance Component %CV estimates

	%CV estimate		
	Non-amplified	IHC 2+	Amplified
Site-to-site	9.0%	12.2%	15.8%
Day-to-day	8.4%	12.2%	13.6%
Residual error	8.4%	12.2%	13.1%

g. Observer-to-Observer Reproducibility

The objective of the study was to evaluate observer-to-observer variation on the *HER2*/CEN-17 ratio when specimens stained by *HER2* CISH pharmDx™ Kit was read by three different observers:

- Observer 1 – US certified pathologist (original observer)
- Observer 2 – Additional US certified pathologist
- Observer 3 – Internal observer Dako Denmark A/S

The observer-to-observer study was performed as a blinded, randomized, observer study using formalin-fixed, paraffin-embedded (FFPE) human breast cancer specimens that were a subset of the FFPE breast cancer specimens stained in the method comparison study. The 100 specimens (one specimen per slide) included in the observer-to-observer study were selected using the following rules:

- 1) Out of the 295 consecutive specimens with successful staining, 50 specimens were randomly selected (47 non-amplified, 3 amplified).
- 2) Out of the 57 additional specimens (with IHC *HER2* 2+) with successful staining, 50 specimens were randomly selected (39 non-amplified, 11 amplified).

Furthermore, out of the 100 specimens there are 5 with a ratio within the borderline range (1.8-2.2) based on Observer 1 results.

Table 6 – Observer-to-Observer Reproducibility Results

		Observer 2 Status		Total
		Non-amplified	Amplified	
Observer 1 Status	Non-amplified	85	1	86
	Amplified	7	7	14
Total		92	8	100

Summary of Safety and Effectiveness Data (SSED)

		Observer 3 Status		Total
		Non-amplified	Amplified	
Observer 1 Status	Non-amplified	84	2	86
	Amplified	2	12	14
Total		86	14	100

		Observer 3 Status		Total
		Non-amplified	Amplified	
Observer 2 Status	Non-amplified	86	6	92
	Amplified	0	8	8
Total		86	14	100

Each of the 100 selected specimens originally evaluated in the Method Comparison study was evaluated by two additional observers for a total of three observations per slide. The %CV for the *HER2*/CEN-17 ratio was calculated for each specimen across all three observers. The mean %CV for the *HER2*/CEN-17 ratio for all 100 specimens is 12.4% while for the 87 non-amplified samples it was 10.9% and for the 13 amplified samples, 22.6%.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The safety and effectiveness of *HER2* CISH pharmDx™ Kit has been evaluated in the Dako sponsored clinical study: *Comparative study of HER2 CISH pharmDx™ Kit with the PathVysion® HER-2 DNA Probe Kit and HER2 FISH pharmDx™ Kit*. This study provided comparative data between *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit, an FDA approved, and commercially available fluorescence In-situ hybridization (FISH) method (P980024). Data from this clinical study was the basis of this PMA approval decision. In order to support a prognostic claim a method comparison study between *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit (P040005) was performed. A summary of the clinical study is presented below.

A. Study Design

The design of the study was comparative, where *HER2* gene status achieved by the *HER2* CISH pharmDx™ Kit was compared to *HER2* gene status obtained by the PathVysion® *HER-2* DNA Probe Kit (FISH) and to the *HER2* FISH pharmDx™ Kit

on formalin-fixed and paraffin-embedded (FFPE) histological sections from breast cancer specimens. Only complete tissue sections were stained and evaluated in the study.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the Comparative study of *HER2* CISH pharmDx™ Kit with the PathVysion® *HER-2* DNA Probe Kit and *HER2* FISH pharmDx™ Kit study was limited to specimens that met the following inclusion criteria:

- A confirmed pathology diagnosis of invasive breast cancer
- Adequate tissue specimen was available (a minimum of 7 slides were needed)
- The sample was residual
- The HercepTest™ IHC score, as per the manufacturer's Package Insert, was known for the specimen
- Fixation history of the specimen was known

Specimens were not permitted to enroll in the Comparative study of *HER2* CISH pharmDx™ Kit with the PathVysion® *HER-2* DNA Probe Kit and *HER2* FISH pharmDx™ Kit study if they met any of the following exclusion criteria:

- Inadequate or no existing tissue specimen for the entire study available
- Missing tissue specimen
- Tissue specimen was from a core or needle biopsy
- A specimen could be excluded from some or all of the analysis if either CISH or FISH fails in both of the two attempts
- Tissue sections from specimen that do not contain invasive tumor
- Specimen was not fixed in neutral buffered formalin

2. Follow-up Schedule

Not applicable.

3. Clinical Endpoints

The primary objective of the study was to investigate the concordance between the *HER2* gene status obtained using *HER2* CISH pharmDx™ Kit, and the *HER2* gene status obtained using PathVysion® *HER-2* DNA Probe Kit (FISH) on serial sections of the same breast cancer specimens.

The secondary objective of the study was to investigate the concordance between the *HER2* gene status obtained using Dako *HER2* CISH pharmDx™ Kit, and the *HER2* gene status obtained using Dako *HER2* FISH pharmDx™ Kit on serial sections of the same breast cancer specimens.

Summary of Safety and Effectiveness Data (SSED)

The tertiary objective was to compare slide evaluation times with the *HER2* CISH pharmDx™ Kit and the PathVysion® *HER-2* DNA Probe Kit (FISH).

B. Description of Samples in the PMA Cohort

Three hundred sixty-five invasive breast cancer specimens selected in chronological order were included in the study. For the first 304 specimens the incoming specimens were selected regardless of the HercepTest™ score. For the next 61 specimens only those with a HercepTest™ IHC 2+ score were selected in order to enrich the study population with IHC 2+ cases. From these 365 specimens, consecutive sections from 350 breast cancer specimens were successfully analyzed by both *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH).

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for a *HER2* testing study performed in the US at a reference laboratory. A total of 365 FFPE breast cancer specimens from different patients were enrolled in this comparative study. The specimens were selected chronologically as they arrived at the US reference laboratory for *HER2* status determination. As the specimens were consecutive specimens coming in to central lab from hospitals across the United States it is expected that the specimens in the study represent characteristics of the US breast cancer population.

D. Safety and Effectiveness Results

1. Method Comparison Study - *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit

Test results were obtained from 365 breast cancer specimens selected in chronological order. For the first 304 specimens the incoming specimens were selected regardless of the HercepTest™ score. For the next 61 specimens only those with a HercepTest™ IHC 2+ score were selected in order to enrich the study population with IHC 2+ cases.

Table 7: Numbers of valid and missing test results for the comparison between *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) assays.

Type			HercepTest	CISH	PathVysion
Consecutive	N	Valid	304	295	300
		Missing	0	9	4
IHC2+	N	Valid	61	57	61
		Missing	0	4	0

Summary of Safety and Effectiveness Data (SSED)

The numbers of valid and missing test results are presented in Table 7. For *HER2* CISH pharmDx™ assay 13 results were not available and for PathVysion *HER-2* DNA Probe Kit (FISH) 4 specimens were without a final test result.

Cross Tabulations of *HER2* Status for All Valid Specimens

For the comparison of *HER2* status obtained by the *HER2* CISH pharmDx™ Kit and the PathVysion *HER-2* DNA Probe Kit (FISH) a total of 350 valid specimens were eligible

For *HER2* CISH staining, two (2) out of four (4) of the missing test results were due to 2 test failures with regard to staining quality. The last two missing CISH result were due to lack of patient sample to perform repeat test (see Table 8).

Table 8: Case processing summary for *HER2* CISH pharmDx™ Kit versus PathVysion FISH *HER2* gene status – all cases.

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CISH status * PathVysion FISH status	350	95.9%	15	4.1%	365	100.0%

For these 350 specimens, the HercepTest™ IHC scores distribution of amplified and non-amplified specimens as determined by *HER2* CISH pharmDx™ Kit is shown in Table 9.

Table 9: Cross tabulation of HercepTest™ IHC score and CISH *HER2* gene status

		HercepTest IHC score				Total
		0	1+	2+	3+	
CISH status	non-amplified	98	108	95	5	306
	amplified	0	1	17	26	44
Total		98	109	112	31	350

By cross tabulation of the *HER2* status an overall agreement at 97.7% and a Kappa value at 0.90 were found (Table 10). Positive agreement was 90.9% with lower and upper 95% confidence intervals based on the binomial distribution at 79.8% and 96.9%, respectively. Negative agreement was 98.7% with lower and upper 95% confidence intervals at 96.9% and 99.6%, respectively. McNemar's test for a systematic bias between *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) assays revealed a non-significant two-tailed p value at 1.00, showing that no bias between the assays was present.

Summary of Safety and Effectiveness Data (SSED)

Table 10: Cross tabulation of CISH *HER2* gene status versus PathVysion FISH *HER2* gene status.

		PathVysion FISH status		Total
		non-amplified	amplified	
CISH status	non-amplified	302	4	306
	amplified	4	40	44
Total		306	44	350

Overall agreement: $(342/350 \times 100) = 97.7\%$ (CI95: 95.7%; 98.9%)

Positive agreement: $(40/44 \times 100) = 90.9\%$ (CI95: 79.8%; 96.9%)

Negative agreement: $(302/306 \times 100) = 98.7\%$ (CI95: 96.9%; 99.6%)

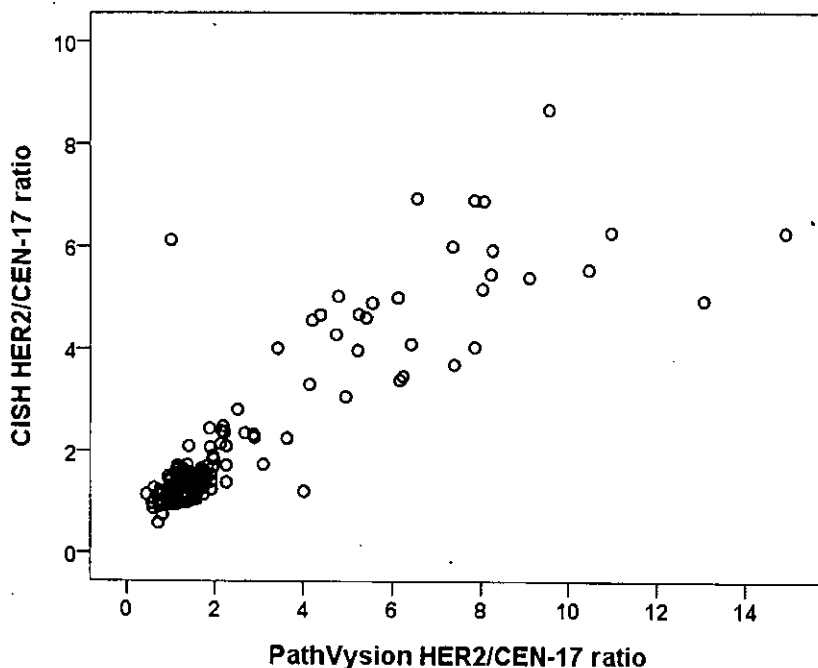


Figure 1. Plot of *HER2*/CEN-17 ratios for Dako *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH)

Linear regression of the logarithmically transformed *HER2*/CEN-17 ratios revealed a correlation coefficient at 0.90 and a slope of 0.71

From the cross tabulation of specimens analyzed with *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH), it is observed that eight specimens do not get the same *HER2* gene status. Below, in Table 11, a short data summary is provided with the HercepTest™ IHC scores, *HER2*/CEN-17 ratios, and *HER2* status for these specimens. These eight specimens represent HercepTest™ IHC scores of 2+ or 3+. Five of the specimens (1001, 1098, 1401, 1411, 2056) have CISH *HER2*/CEN-17 ratios close to or within the borderline area of 1.8 to 2.2 in which the obtained results should be interpreted with caution. Thorough analysis of the discrepant cases have lead to the inclusion of several precautions in the instruction for use of the product (see safety conclusion).

Summary of Safety and Effectiveness Data (SSED)

Table 11: Specimens that disagree in CISH versus PathVysion FISH *HER2* gene status.

	Specimen	HercepTest IHC score	CISH <i>HER2</i> /CEN-17 ratio	CISH status	PathVysion <i>HER2</i> /CEN-17 ratio	PathVysion FISH status
1	DAKO 09-1001	2+	1.72	non-amplified	2.27	amplified
2	DAKO 09-1004	3+	1.21	non-amplified	4.02	amplified
3	DAKO 09-1020	3+	6.13	amplified	1.00	non-amplified
4	DAKO 09-1098	3+	1.74	non-amplified	3.10	amplified
5	DAKO 09-1401	2+	2.07	amplified	1.90	non-amplified
6	DAKO 09-1411	2+	2.09	amplified	1.41	non-amplified
7	DAKO 09-1460	2+	1.38	non-amplified	2.27	amplified
8	DAKO 09-2056	2+	2.44	amplified	1.88	non-amplified

Cross Tabulations of *HER2* Status for IHC 2+ Specimens Only

In the current study a total of 112 specimens with a HercepTest™ IHC 2+ score were eligible for *HER2* status comparison by the *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH).

Cross tabulation of the *HER2* status revealed an overall agreement for *HER2* status at 95.5% (Table 12) and a Kappa value at 0.82. Positive agreement was 87.5% with lower and upper 95% confidence intervals based on the binomial distribution at 65.6% and 97.3%, respectively. Negative agreement was 96.9% with lower and upper 95% confidence intervals at 91.9% and 99.1%, respectively. The two-tailed confidence intervals are based on a binomial distribution. McNemar's test for a systematic bias between CISH and PathVysion assays revealed a non-significant two-tailed p-value at 1.00, showing that no bias was present.

Table 12: Cross tabulation of CISH *HER2* gene status versus PathVysion FISH *HER2* gene status – IHC 2+ specimens only.

		PathVysion FISH status		Total
		non-amplified	amplified	
CISH status	non-amplified	93	2	95
	amplified	3	14	17
Total		96	16	112

Overall agreement: $(107/112 \times 100) = 95.5\%$ (CI95: 90.5%; 98.3%)

Positive agreement: $(14/16 \times 100) = 87.5\%$ (CI95: 65.6%, 97.3%)

Negative agreement: $(93/96 \times 100) = 96.9\%$ (CI95: 91.9%, 99.1%)

HER2 CISH Success Rate

Of the 365 specimens enrolled, the *HER2* CISH pharmDx™ test was not performed for one specimen. For a total of 364 initial CISH tests performed, a *HER2*/CEN-17 ratio could be obtained in 352 tests. Therefore, the CISH success rate was $(352/364 \times 100)\%$, i.e. 96.7%.

Conclusion on *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) Comparison Study

The clinical utility of *HER2* CISH pharmDx™ Kit has been investigated using 365 invasive breast cancer specimens that were analyzed by *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH). Specimens were enrolled consecutively as they arrived at the US reference laboratory for *HER2* status analysis and the study population was enriched by HercepTest™ IHC 2+ specimens to challenge testing close to the cut-off value. Consecutive sections from 350 breast cancer specimens were successfully analyzed by both *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH). The overall agreement between *HER2* status obtained by *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) for 350 breast cancer specimens was 97.7% (CI95 limits 95.7%; 98.9%), with a Kappa of 0.90 indicating almost perfect agreement. Positive agreement was 90.9% (CI95 limits 79.8%; 96.9%), whereas, negative agreement was 98.7% (CI95 limits 96.9%; 99.6%). There was no systematic bias between the two tests. The acceptance criteria set prior to the clinical study were based on the positive and negative *HER2* status agreements including the lower bound confidence intervals for positive and negative agreements previously obtained in a comparison between *HER2* FISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) assays. The agreements and confidence limits obtained are summarized together with the acceptance criteria in the table below.

Table 13: Acceptance criteria defined prior to study initiation and the specific agreements obtained for the comparison between *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH).

	Positive agreement		Negative agreement	
	Positive Agreement	Lower 95% CI limit	Negative agreement	Lower 95% CI limit
Acceptance criteria	86%	77%	97%	94%
CISH versus PathVysion	90.9%	79.8%	98.7%	96.9%

The positive and negative agreements including the lower 95% confidence intervals for the comparison between *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) met the acceptance criteria defined prior to the initiation of the study. These study results support the predictive claim for *HER2* CISH pharmDx™ Kit indicated as an aid in the assessment of patients for whom Herceptin™ is being considered.

Of the 364 specimens for which a CISH assay was initiated, a *HER2*/CEN-17 ratio could be obtained in 352 tests resulting in a CISH success rate of 96.7%.

2. Method Comparison Study - *HER2* CISH pharmDx™ Kit and Dako *HER2* FISH pharmDx™ (Prognostic value of *HER2* CISH pharmDx™ Kit)

In order to link the prognostic value of *HER2* FISH pharmDx™ Kit to the *HER2* CISH pharmDx™ Kit the concordance between test results obtained with these assays was investigated. The prognostic value of *HER2* FISH pharmDx™ Kit was previously shown based on the data in the DBCG (Danish Breast Cancer Cooperative Group) study 89D that analyzed *HER2* status using Dako *HER2* FISH pharmDx™ Kit. In the DBCG 89D study test results from 649 patient specimens were available for multivariate analysis, 417 had a *HER2*/CEN-17 ratio below 2.0 (normal or non-amplified *HER2* gene status) and 232 had a *HER2*/CEN-17 ratio above or equal to 2.0 (amplified *HER2* gene status).

The results showed that patients with *HER2* amplification had more severe prognosis, as they were older, had higher malignancy grade, greater tumor size, more tumor positive lymph nodes, more abnormal TOP2A status and higher *HER2* IHC score. *HER2* amplification had no significant prognostic value when tested in all patients (N=649) using the multivariate Cox proportional hazards model. However, *HER2* amplification had an independent prognostic value for both recurrence free survival (RFS): HR=1.42 (1.08-1.85), p=0.011, and overall survival (OS): HR=1.40 (1.07-1.85), p=0.015, when testing in the sub-group of node-positive patients (n=423). Therefore, it was concluded that *HER2* amplification had an independent prognostic value for both RFS and OS.

The concordance between *HER2* FISH pharmDx™ Kit and *HER2* CISH pharmDx™ Kit assays was investigated in a clinical study comprising 365 invasive breast cancer specimens collected in chronological order. The first 304 specimens were selected regardless of the HercepTest™ score, and the next 61 specimens were selected based on a HercepTest™ IHC 2+ score in order to enrich the study population with IHC 2+ cases.

A total of 348 specimens with a valid test result for both assays were available for the concordance analysis between *HER2* CISH pharmDx™ Kit and the *HER2* FISH pharmDx™ Kit. The cross tabulation of the *HER2* status for the two Dako assays is shown in Table 14.

Summary of Safety and Effectiveness Data (SSED)

Table 14. Cross tabulation HER2 status obtained using the *HER2* CISH pharmDx™ Kit and the *HER2* FISH pharmDx™ Kit.

DAKO CISH vs. FISH		FISH status		Total
		non-amplified	amplified	
CISH status	non-amplified	301	3	304
	amplified	3	41	44
Total		304	44	348

Overall agreement: $(342/348 \times 100) = 98.3\%$ (CI95: 96.5%; 99.3%)

Positive agreement: $(41/44 \times 100) = 93.2\%$ (CI95: 82.9%; 98.0%)

Negative agreement: $(301/304 \times 100) = 99.0\%$ (CI95: 97.4%; 99.7%)

The cross tabulation presented in Table 14 showed an overall agreement between the two assays of 98.3% (CI95: 96.5%; 99.3%). The positive and negative agreement were 93.2% (CI95: 82.9%; 98.0%) and 99.0% (CI95: 97.4%; 99.7%) respectively. Likewise, a Kappa coefficient of 0.92 underlined the high agreement between *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit assays.

Conclusion on *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit Comparison

A high overall agreement between the two Dako assays of 98.3% was obtained in this study, with positive and negative agreements at 93.2% and 99.0%, respectively. The acceptance criteria set prior to study initiation were identical to the criteria for the *HER2* CISH pharmDx™ Kit and PathVysion *HER-2* DNA Probe Kit (FISH) comparison. In Table 15 these acceptance criteria are listed together with the agreements obtained for the *HER2* status comparison between *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit.

Table 15. Acceptance criteria defined prior to study initiation for the *HER2* status comparison between *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit.

	Positive agreement	Positive agreement lower 95% CI limit	Negative agreement	Negative agreement lower 95% CI limit
Acceptance criteria	86%	77%	97%	94%
CISH versus FISH	93.2%	82.9%	99.0%	97.4%

Conclusion on Studies to Demonstrate Prognostic Claim for *HER2* CISH pharmDx™ Kit

A large number of studies have confirmed the prognostic values of *HER2* status in breast cancer, both with respect to overexpression of the protein and amplification of the gene. One of these studies is the DBCG 89D study where *HER2* amplification measured by the *HER2* FISH pharmDx™ Kit showed to have an independent prognostic value for both RFS and OS when testing in node positive breast cancer patients. In order to transfer the prognostic claim from the *HER2* FISH pharmDx™ Kit (PMA P040005) to the *HER2* CISH pharmDx™ Kit a concordance study demonstrating overall agreement at 98.3% between the two

Summary of Safety and Effectiveness Data (SSED)

pharmDx™ Kits has been performed. Acceptance criteria based on positive and negative agreements as well as the lower 95% confidence limits for these were met.

Based on the result from the DBCG 89D study and the comparison between *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit it is justified that the prognostic claim of *HER2* FISH pharmDx™ Kit is transferred to *HER2* CISH pharmDx™ Kit.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The adverse effects of this device are based on data collected in clinical study conducted to support PMA approval as described above. As a diagnostic test, *HER2* CISH pharmDx™ assay involves testing on formalin-fixed, paraffin-embedded human breast cancer tissue sections. These tissue sections are routinely removed for breast cancer diagnosis. The test, therefore, presents no additional safety hazard to the patient being tested if the following precautions, based on the preclinical and clinical studies, are followed as outlined in the instructions for use:

- If a specimen includes cluster of blue signals (amplification of CEN-17) that makes it difficult to count the red *HER2* signals and thereby interpret the staining, it is recommended to note the score and the staining pattern for reference. The user then needs to refer to other test methods (e.g. FISH or IHC) to make a final conclusion on *HER2* status.
- Due to potential heterogeneity of the *HER2* signal distribution, it is important to perform a thorough scanning of the complete CISH stained specimen to evaluate signal distribution before selecting the area for signal enumeration.
- The appearance of "Giant cells" within the tumor area should be noted and interpreted with caution since these cells can potentially affect the status of the specimen.

B. Effectiveness Conclusions

Results of the non-clinical and clinical studies using *HER2* CISH pharmDx™ Kit on formalin-fixed, paraffin-embedded human breast cancer tissue sections with varying levels of gene amplification demonstrated:

Summary of Safety and Effectiveness Data (SSED)

HER2 status determined in the comparative clinical study using *HER2* CISH pharmDx™ Kit showed an overall agreement to that obtained by PathVysion *HER-2* DNA Probe Kit (FISH) at 98% (95% CI: 96%-99%), with positive and negative agreements at 91% and 99%, respectively.

Likewise, determination of HER2 status when using *HER2* CISH pharmDx™ Kit and *HER2* FISH pharmDx™ Kit showed an overall agreement at 98.3% (95% CI: 96.5% - 99.3%) with positive and negative agreements at 93% and 99%, respectively. Based on the presentation of data in the DBCG 89D study using *HER2* FISH pharmDx™ Kit the prognostic claim from the *HER2* FISH pharmDx™ Kit can be transferred to the *HER2* CISH pharmDx™ Kit.

Analysis of the data from the clinical reproducibility study using variance component models revealed that the *HER2* CISH pharmDx™ Kit demonstrated day-to-day, site-to-site reproducibility $\leq 12\%$ CV for non-amplified (normal) and *HER2* IHC 2+ (HercepTest™) specimens and $\leq 16\%$ CV for amplified specimens.

Analysis of data from the observer-to-observer reproducibility study showed a CV=12.4% for all specimens. This could be further divided into a CV=10.9% for non-amplified and 22.6% for amplified specimens defined by the consensus status.

The success rate for *HER2* CISH pharmDx™ Kit was determined to be 96.7% in the comparative clinical study.

C. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the intended use.

XIII. CDRH DECISION

CDRH issued an approvable letter on November 30, 2011. The final conditions of approval cited in the approval order are described in the approval order.

The applicant's manufacturing facility was inspected and found to be in compliance with the device Quality Systems (QS) regulation (21 CFR 820) on August 11, 2011.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.